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For:	ISOTOPICALLY LABELLED, INDOLINONE DERIVATIVES AND PROCESS FOR THEIR PREPARATION	**Dated:**	August 6, 2008

Confirmation No.: 3819

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
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CLAIM OF PRIORITY

Sir:

Applicants in the above-identified application hereby claim the right of priority in connection with Title 35 U.S.C. §119 and in support thereof herewith submit a certified copy of 02078164.7 filed August 1, 2002 in Europe.

Respectfully submitted,

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Dated: August 6, 2008

Peter I. Bernstein

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Bescheinigung

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

Certificate

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Attestation

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécificée à la page suivante.

Patentanmeldung Nr. **Patent application No.** **Demande de brevet n°**

02078164.7 / EP02078164

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP02078164

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

R.C. van Dijk

Anmeldung Nr:
Application no.: 02078164.7
Demande no:

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Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Isotopically labelled indolinone derivatives and process for their preparation

In anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)
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ISOTOPICALLY LABELLED INDOLINONE DERIVATIVES AND PROCESS FOR THEIR PREPARATION

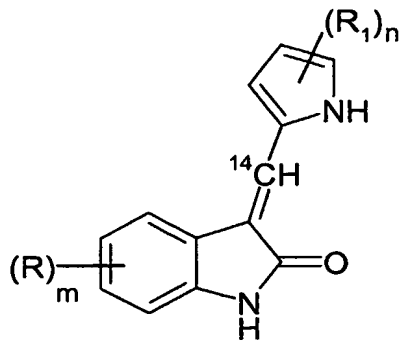
The present invention relates to indolinone derivatives and, more particularly, it relates to the above compounds isotopically labelled with carbonium 14 [^{14}C], and to a process for their preparation.

Several indolinone derivatives are known in the art as therapeutic agents.
Particularly relevant, among them, are certain (1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl-1H-pyrrole derivatives, hereinafter shortly referred to as indolylidene-methyl-pyrroles, disclosed by Sugen Inc. in a variety of patents and patent applications, among which are US 5,880,141, US 5,792,783, WO 99/61422 and WO 01/37820, herewith incorporated by reference.
By modulating tyrosine kinase signal transduction, the said compounds are useful in therapy for regulating, modulating and/or inhibiting abnormal cell proliferation.

Because of their use in therapy, for instance in the treatment of cancer, the possibility of their preparation as isotopically labelled carbonium 14 [^{14}C] compounds is of utmost importance for absorption, distribution, metabolism and excretion (ADME) studies.

From the above, we have now found a new class of indolylidene-methyl-pyrroles being isotopically labelled with [^{14}C] at the methylidene moiety.

It is therefore a first object of the present invention a compound of general formula (I) below:



(I)

wherein

each R group is, at one or more of the positions 4, 5, 6 and 7 of the indolinone ring and independently from each other, a straight or branched C_1-C_4 alkyl or alkoxy group or a halogen atom;

each R_1 group is, the same or different, a C_1-C_4 alkyl or a group of general formula $-(CH_2)_pCO_2R'$ or $-(CH_2)_p-CONR'R"$ wherein p is 0, 1 or 2 and R' and R" are selected, each independently, from hydrogen or straight or branched C_1-C_4 alkyl optionally substituted by hydroxy or, taken together with the nitrogen atom to which they are attached, R' and R" may form a pyrrolidino, piperidino or morpholino group;

m is 0 or an integer from 1 to 4;

n is 0 or an integer from 1 to 3;

or pharmaceutically acceptable salts thereof.

As clearly reported in formula (I), labelling with ^{14}C occurs at the methylidene moiety bridging the indolinone with the pyrrole ring.

The compounds of formula (I) may have asymmetric carbon atoms and may therefore exist either as racemic mixtures or as individual optical isomers. In addition, the double bond in general formula (I) between the carbon atom in position 3 of the indolinone ring and the labelled [^{14}C] atom, may be such to give rise to any one of the cis (Z) or trans (E) isomers.

From the foregoing and unless otherwise provided, all of the optical or geometrical isomers as well as mixtures

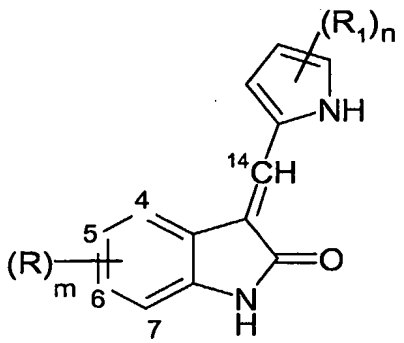
thereof, have to be intended as comprised within the scope of the present invention.

Unless otherwise provided, in the present description, with the terms straight or branched C_1-C_4 alkyl or alkoxy group we intend, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy and tert-butoxy.

With the term halogen atom we intend a fluorine, chlorine, bromine or iodine atom.

Pharmaceutically acceptable salts of the compounds of formula (I) are the acid addition salts with inorganic or organic acids, e.g. nitric, hydrochloric, hydrobromic, sulphuric, perchloric, phosphoric, acetic, trifluoroacetic, propionic, glycolic, lactic, oxalic, malonic, malic, maleic, tartaric, citric, benzoic, cinnamic, mandelic, methanesulfonic, isethionic and salicylic acid, as well as the salts with inorganic or organic bases, e.g. alkali or alkaline-earth metals, especially sodium, potassium, calcium or magnesium hydroxides, carbonates or bicarbonates, acyclic or cyclic amines, preferably methylamine, ethylamine, diethylamine, triethylamine or piperidine.

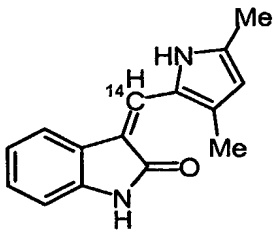
As formerly indicated, the indolinone derivatives of the invention may be further substituted in one or more of the positions 4, 5, 6 and 7, according to the numbering system below:



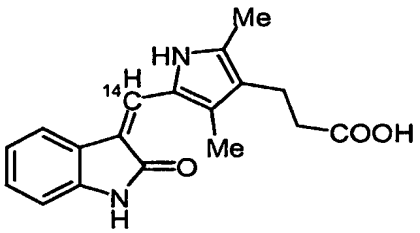
(I)

Preferably, the compounds of the invention may be represented by the above general formula (I) wherein the pyrrole ring is substituted by one or more groups such as, for instance, methyl, carboxy, ethoxycarbonyl, carboxyethyl, N,N-diethyl-aminocarbonyl, and the like.

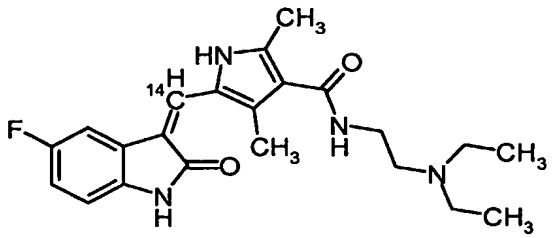
Even more preferably, the compounds of the invention are selected from 3-[(3,5-dimethyl-1H-pyrrol-2-yl)[^{14}C]methylene-1,3-dihydro-2H-indol-2-one (hereinafter shortly referred to as [^{14}C]SU-5416); 5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-propionic acid (hereinafter shortly referred to as [^{14}C]SU-6668); and N-[-(2-diethylamino)ethyl]-5-[(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-ylidene)[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide (hereinafter shortly referred to as [^{14}C]SU 11248), of formula:



([^{14}C]SU 5416)



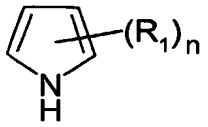
([^{14}C]SU 6668)



([^{14}C]SU 11248)

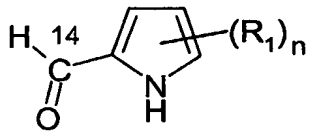
As formerly indicated, it is another object of the invention a process for preparing the compounds of formula (I), which process comprises:

a) reacting dimethyl-[^{14}C]formamide with a suitable pyrrole derivative of formula (II), in the presence of diphosphoryl-chloride



(II)

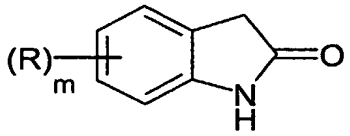
wherein R_1 and n are as above defined, so as to obtain a compound of formula (III)



(III)

and optionally converting a compound of formula (III) into another compound of formula (III);

b) reacting under basic conditions the compound of formula (III) with an oxindole derivative of formula (IV)



(IV)

wherein R and m are as above defined, so as to obtain a compound of formula (I) and, optionally converting it into another compound of formula (I) and/or into a pharmaceutically acceptable salt thereof.

The above process is particularly advantageous as it enables the selective preparation of a variety of compounds of formula (I) isotopically labelled with [^{14}C], being optionally substituted with several R and R_1 groups on both the indolinone and/or pyrrole moieties.
In addition, it enables the preparation of the desired derivatives in high yields and with a high degree of radiochemical purity.

According to step (a) of the process, dimethyl-[^{14}C]formamide is reacted with a proper pyrrole derivative,

either substituted or unsubstituted by R_1 groups, as formerly indicated. The reaction is carried out under inert atmosphere, e.g. nitrogen or argon, in the presence of diphosphoryl chloride, at a temperature from about 0°C to about room temperature and for a time of about 40 minutes.

As formerly indicated, the compounds of formula (III) thus prepared may be conveniently converted into others compounds of formula (III), for instance by transforming a given R' group into another R' group. As an example, a compound of formula (III) bearing an ester R_1 group, e.g. $-(CH_2)_pCO_2R'$ with R' as alkyl, may be conveniently converted into the corresponding carboxylic acid derivative wherein R' is hydrogen.

The above reaction may be either carried out subsequently to the preparation of the compound of formula (III) or, advantageously, in one pot without the need of isolating any intermediate derivative. Any of the above conversions may be carried out according to well known methods.

As an example, the conversion of an ester group into the corresponding carboxylic acid derivative may be easily accomplished through basic hydrolysis, for instance in the presence of potassium hydroxide under water/methanol refluxing conditions.

Likewise, any of the above derivatives of formula (III) bearing a R_1 group corresponding to $-(CH_2)_pCO_2H$ may be also converted, whenever desired, into the corresponding carboxamido derivatives $-(CH_2)_p-CONR'R"$. Also the above reaction is performed according to conventional amidation conditions, for instance by reacting the proper carboxylic acid derivative of formula (III) with the proper amino derivative, in the presence of benzotriazol-1-ylotris(dimethylamino)phosphonium hexafluorophosphate (BOP) and of a tertiary amine, e.g. triethylamine.

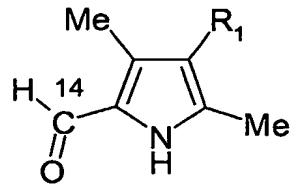
The reaction may occur in the presence of a suitable solvent, e.g. dimethylformamide, and at room temperature.

According to step (b) of the process, any of the above compounds of formula (III) is reacted, under basic conditions, with a suitable indolinone derivative of formula (IV). This condensation reaction is carried out according to conventional methods, in the presence of catalytic amounts of a suitable base, e.g. pyrrolidine, and in a suitable solvent, e.g. ethanol, at refluxing conditions and for a suitable time, e.g. from about 30 to about 90 minutes.

By working as above reported in step (a) when converting a compound of formula (III) into another derivative of formula (III), also the compounds of formula (I) being obtained in step (b) may be conveniently converted into other derivatives of formula (I).
As an example, any given compound of formula (I) wherein R_1 is an ester group may be converted into the corresponding derivative of formula (I) wherein R_1 may represent a carboxy and/or carboxamido group, as formerly described.
Likewise, the optional salification of a compound of formula (I) or the conversion of its salt into the free compound, as well as the separation of a mixture of isomers into the single isomers, may be all carried out by conventional methods.

The starting dimethyl-[^{14}C]formamide is a commercially available compound and any of the derivatives of formula (II) and (IV) is known or may be prepared according to well-known synthetic methods.

According to a preferred embodiment of the invention, the above process is addressed to the preparation of the aforementioned isotopically [^{14}C] labelled indolinone derivatives SU 5416, SU 6668 and SU 11248.
In this respect, any of the intermediate derivatives of formula (IIIa) below



(IIIa)

wherein R_1 is a hydrogen atom or a group $-(CH_2)_2-CO_2H$, $-CO_2H$, $-CO_2CH_2CH_3$ and $-CONH-(CH_2)_2-N(CH_2CH_3)_2$ is novel and, hence, represents a further object of the invention.

The isotopically [^{14}C] labelled indolinone derivatives of formula (I) may be used in ADME studies according to conventional methods, widely known in the art.

With the aim of better illustrate the present invention, without posing any limitation to it, the following examples are now given.

Example 1

Preparation of 3,5-dimethyl-1H-pyrrole-2-[^{14}C]carbaldehyde

[^{14}C]-DMF (about 740 MBq, 1.045 mmol) was cooled with an ice bath and very slowly added via a syringe with diphosphoryl chloride (DPC) (380 µl; 2.76 mmol). After stirring at about 0°C under nitrogen atmosphere for 10 minutes, 2,4 dimethylpyrrole (130 µl;1.275 mmol) was added to the solution over a period of 10 minutes and the mixture was stirred for 30 minutes at room temperature (rt). At the end of reaction (checked by radio-HPLC on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume) the mixture was cooled at -10°C and a solution of methanol:water 1:5 v:v (3 ml) was introduced into the flask. After adjusting the pH to about 12 by addition of

10% KOH, a white suspension was obtained which was filtered through a D4 sintered-glass filter and washed with water (4x 3 ml). The solid 3,5-dimethyl-1H-pyrrole-2-[^{14}C]carbaldehyde was obtained as a white solid (360 MBq), 95% radiochemically pure. The radiochemical purity of the title compound was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 9.11 minutes) was the same as the retention time of an authentic non-labelled sample. The radiochemical yield of this step was about 49%.

Example 2

Preparation of 3-[(3,5-dimethyl-1H-pyrrol-2-yl)[^{14}C]methylene]-1,3-dihydro-2H-indol-2-one ([^{14}C]SU 5416). 3,5-dimethyl-1H-pyrrole-2-[^{14}C]carbaldehyde (about 360 MBq; 0.48 mmol prepared as described, for instance, in example 1) and oxindole (64.3 mg; 0.48 mmol) were dissolved with ethanol (3 ml). Pyrrolidine (70 µl; 1.71 mmol) was then added and the solution was stirred at reflux for 90 minutes in the dark. The obtained suspension was cooled at rt and filtered through a D4 sintered-glass filter giving a yellow-red solid that was washed with ethanol (4 x 3 ml). After drying, 3-[(3,5-dimethyl-1H-pyrrol-2-yl)[^{14}C]methylene]-1,3-dihydro-2H-indol-2-one ([^{14}C]SU 5416) was obtained (about 194 MBq; 0.261 mmol) 99 % radiochemically pure. The radiochemical purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient

over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 15.4 minutes) was the same as the retention time of an authentic non-labelled sample. The mass spectrum of the title compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m/z 241 of 3-[(3,5-dimethyl-1H-pyrrol-2-yl)[^{14}C]methylene]-1,3-dihydro-2H-indol-2-one and also the protonated molecular ions at m/z 239 of 3-[(3,5-dimethyl-1H-pyrrol-2-yl)methylene]-1,3-dihydro-2H-indol-2-one. The radiochemical yield of this step was about 54%.

Example 3

Preparation of 3-(3,5-dimethyl-2-[^{14}C]formyl-1H-pyrrol-4-yl)-propionic acid

[^{14}C]DMF (about 740 MBq, 1.045 mmol) was cooled with an ice bath and very slowly added via a syringe with DPC (900 µl). After 10 minutes of stirring, the above cooled (ice bath) solution was added with 3-(2,4-dimethyl-1H-pyrrol-3-yl)propanoic acid (213 mg, 1.27 mmol) over 15 minutes under nitrogen then allowed to warm to room temperature and the mixture was stirred for 30 minutes at rt. At the end of reaction, checked by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the mixture was cooled at -10°C, a solution of methanol:water 1:5 v:v (3 ml) was added. After adjusting the pH to about 12 by addition of 45% KOH, the solution was

stirred at 0°C for 30 minutes. The suspension was filtered through a D4 sintered-glass filter obtaining a yellow clear solution, which was added with 10 N HCl up to pH 3.5. The mixture was stirred at 0°C for 30 minutes. The resulting brown suspension was filtered through a D4 sintered-glass filter, the intermediate 3-(3,5-dimethyl-2-[^{14}C]formyl-1H-pyrrol-4-yl)-propionic acid was obtained as a brown solid (213 MBq; 0.383 mmol), 77% radiochemically pure. The radiochemical purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 7.36 minutes) was the same as the retention time of an authentic non-labelled sample. The radiochemical yield of this step was about 29%.

Example 4

Preparation of (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidene[^{14}C] methyl)-1H-pyrrol-3-yl]-propionic acid ([^{14}C]SU 6668).

3-(3,5-dimethyl-2-[^{14}C]formyl-1H-pyrrol-4-yl)-propionic acid (213 MBq; 0.295 mmol, prepared as described, for instance, in example 3) and oxindole (46 mg; 0.35 mmol) were dissolved with ethanol (2 ml) then pyrrolidine (40µl; 0.977 mmol) was added and the solution was stirred at reflux for 90 minutes in the dark. At the end of reaction checked by radio-HPLC(on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous

with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume) the mixture was cooled to rt, evaporated under vacuum, diluited with water (300 ml) and added with 1N HCl up to pH 2. The solution was transferred into a separating funnel and extracted with EtOAc (3 x 100 ml). The collected organic phases were pooled, washed with brine (2 x 100 ml) and after evaporation to dryness under vacuum, the crude (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-3H-indol-3-ylidene[^{14}C] methyl)-1H-pyrrol-3-yl]-propionic acid ([^{14}C]SU 6668) was obtained (171.5 MBq; 0.309 mmol) 84% radiochemically pure. The purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 12.5 minutes) was the same as the retention time of an authentic non-labelled sample. The crude [^{14}C]SU 6668 with a radiochemical purity of about 84% (prepared as above described) was dissolved in a mixture DMSO: mobile phase A (1:2 by volume) up to a concentration of about 6.5 mg/ml and the solution was protected from light.

Aliquots of about 5 ml of the above solution were injected into the preparative HPLC system (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid (A)90:10:0.1 and (B)10:90:0.1 by volume, isocratic for 25 minutes at 75%A-25%B, linear gradient over 5 minutes up to 100%B and 10 minutes of isocratic elution at 100%B, detection wavelength = 254 nm). The real time UV-profile plot of the run was followed by sight to identify the [^{14}C]SU 6668 peak. The column eluate corresponding to the pure [^{14}C]SU 6668 was

collected in a glass flask protected from light. The fractions containing the compound were combined and acetonitrile was removed by evaporation. The acidic aqueous solution was transferred into a separating funnel and extracted with EtOAc (1x200 ml). The organic phase was separated, washed with brine (1x200 ml) and after solvent evaporation, [^{14}C]SU 6668 was obtained (98.23 MBq; 0.177 mmol) 99% radiochemically pure. The radiochemical purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 12.5 minutes) was the same as the retention time of an authentic non-labelled sample. The mass spectrum of the title compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m/z 311 amu of (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-3H-indol-3-ylidene[^{14}C]methyl)-1H-pyrrol-3-yl]-propionic acid and also at m/z 309 amu of (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-3H-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid. The radiochemical yield of this step including the purification was about 46%.

Example 5

Preparation of 5-[^{14}C]formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid.

[^{14}C]-DMF (about 740 MBq, 1.309 mmol) was cooled with an ice bath and very slowly added via syringe with DPC (500 µl). After 10 minutes of stirring, the above cooled (ice bath) solution was added with ethyl 2,4-dimethyl-1H-pyrrole-3-

carboxylate (278 mg, 1.66 mmol) over 15 minutes under nitrogen and then allowed to warm to rt. After 30 minutes a check of the reaction mixture (checked by radio-HPLC on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume) showed the complete disappearance of [^{14}C]-DMF. The brown solution was cooled again (ice bath), diluted with a mixture of H2O:MeOH (5:1 by volume; 1 ml), transferred into a cooled (ice bath) round-bottomed flask, added with further H2O:MeOH (5:1 by volume; 4 ml) and adjusted to pH~7 by adding 10% KOH . After introduction of an additional amount of 45% KOH (800 µl) into the reaction flask, the ice bath was removed and the white-yellowish suspension was heated at reflux for 4 hours. After cooling to rt, a clear yellow solution with traces of a brown oil on the surface was obtained. The mixture was adjusted to pH <4 by adding 10% HCl under vigorous stirring obtaining an orange-brown suspension which was filtered through a sintered-glass filtering funnel. The brown solid residue was washed in suspension with 5% HCl (2 × 6 ml) and water until neutral colourless washings were collected (9 × 7 ml). The yellow solid residue was dissolved in a mixture of EtOH:MeOH:DMF for total activity determination and analytical checks. After solvent evaporation to dryness under vacuum, 5-[^{14}C]formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (492 MBq) was obtained > 92% radiochemically pure and used in the next step without further purification. The radiochemical purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5

minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 6.6 minutes) was the same as the retention time of an authentic non-labelled sample. The radiochemical yield of the step was about 66%.

Example 6

Preparation of N-[2-(diethylamino)ethyl]-5-[^{14}C]formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide.

BOP (1 g, 2.26 mmol), TEA (480 µl, 3.43 mmol) and N,N-diethylethane-1,2-diamine (360 µl, 2.56 mmol) were slowly added under nitrogen with stirring to a cooled (ice bath) solution of 5-[^{14}C]formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (167 mg, 455 MBq, 0.1 mmol, prepared, for example, as described in example 5) in DMF (5 ml). The ice bath was removed and the reaction mixture was stirred at rt for 40 minutes. At the end of the reaction (checked by radio-HPLC on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume) the mixture was diluted with water (200 ml) and added with 10% HCl (40 ml). After 10 minutes stirring, the acidic solution was transferred into a separating funnel and washed with EtOAc (3 x 100 ml). The aqueous phase was adjusted to pH >12 by adding 10% KOH and extracted with EtOAc (3 x 80 ml). The collected organic phases were pooled, washed with brine (3 x 70 ml), dried (Na_2SO_4) and, after filtration, evaporated to dryness under vacuum. After solvent evaporation to dryness under vacuum, N-[2-(diethylamino)ethyl]-5-[^{14}C]formyl-2,4-

dimethyl-1H-pyrrole-3-carboxamide (326 MBq) was obtained > 95% radiochemically pure and used in the next step without further purification. The radiochemical purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 4.9 minutes) was the same as the retention time of an authentic non-labelled sample. The radiochemical yield of this step was about 72%.

Example 7

Preparation of N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide ([^{14}C]SU 11248).

5-Fluoro-1,3-dihydro-2H-indol-2-one (137 mg, 0.91 mmol) was added at rt under nitrogen with stirring to a suspension of N-[2-(diethylamino)ethyl]-5-[^{14}C]formyl-2,4-dimethyl-1H-pyrrole-3-carboxamide(190 mg, 326 MBq, 0.71 mmol, prepared as described, for example, in example 6) in EtOH (3 ml). A brown clear solution was obtained and, after addition of pyrrolidine (100 µl, 1.2 mmol), the reaction mixture was refluxed for 30 minutes. At the end of reaction (checked by radio-HPLC on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume) the mixture was cooled to rt, evaporated under vacuum, diluted with water (300 ml) and added with 10%HCl (50 ml). The obtained clear brown

solution was washed with EtOAc (5 x 80 ml), adjusted to pH >12 by adding 10% KOH and extracted with EtOAc (7 x 50 ml). The collected organic phases were pooled, washed with brine (3 x 70 ml) and concentrated under vacuum for activity determination and analytical checks. The solution was evaporated to dryness under vacuum obtaining N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide ([^{14}C]SU 11248)(240 MBq) as a yellow-orange solid > 97% radiochemically pure. The purity was assessed by radio-HPLC (on C-18 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 90:10:0.1 to 10:90:0.1 by volume, linear gradient over 15 minutes and 5 minutes of isocratic elution, detection wavelength = 255 nm, radiometric detection = homogeneous with a 500 µl cell and scintillation cocktail to HPLC effluent ratio of 2:1 by volume), the retention time of title compound (Rt = 9.8 minutes) was the same as the retention time of an authentic non-labelled sample. The mass spectrum of the title compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m/z 411 amu of N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide and also at m/z 409 amu of N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide. The radiochemical yield of this step was about 74%.

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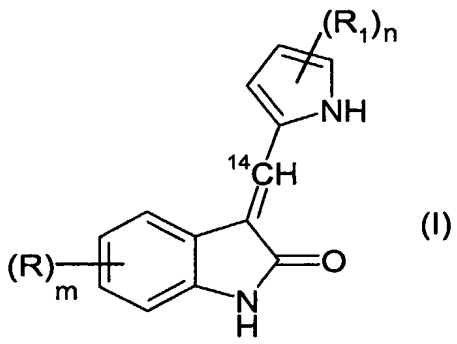
CLAIMS

1. A compound of general formula (I) below:



(I)

wherein
each R group is, at one or more of the positions 4, 5, 6 and 7 of the indolinone ring and independently from each other, a straight or branched C_1-C_4 alkyl or alkoxy group or a halogen atom;
each R_1 group is, the same or different, a C_1-C_4 alkyl or a group of general formula $-(CH_2)_pCO_2R'$ or $-(CH_2)_p-CONR'R"$ wherein p is 0, 1 or 2 and R' and R" are selected, each independently, from hydrogen or straight or branched C_1-C_4 alkyl optionally substituted by hydroxy or, taken together with the nitrogen atom to which they are attached, R' and R" may form a pyrrolidino, piperidino or morpholino group;
m is 0 or an integer from 1 to 4;
n is 0 or an integer from 1 to 3;
or pharmaceutically acceptable salts thereof.

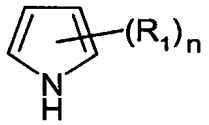
2. A compound according to claim 1 wherein the pyrrole ring is substituted by one or more of the groups selected from methyl, carboxy, ethoxycarbonyl, carboxyethyl or N,N-diethyl-aminocarbonyl.

3. A compound according to claim 1 which is 3-[(3,5-dimethyl-1H-pyrrol-2-yl)[^{14}C]methylene-1,3-dihydro-2H-indol-2-one; 5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-propionic

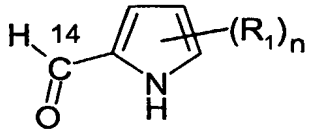
acid; or N-[-(2-diethylamino)ethyl]-5-[(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-ylidene)[^{14}C]methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide.

4. A process for preparing a compound of formula (I) according to claim 1 which process comprises:

a) reacting dimethyl-[^{14}C]formamide with a suitable pyrrole derivative of formula (II), in the presence of diphosphoryl-chloride

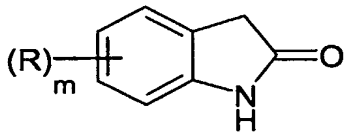

(II)

wherein R_1 and n are as defined in claim 1, so as to obtain a compound of formula (III)


(III)

and optionally converting a compound of formula (III) into another compound of formula (III);

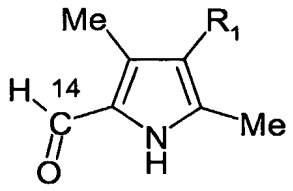
b) reacting under basic conditions the compound of formula (III) with an oxindole derivative of formula (IV)


(IV)

wherein R and m are as defined in claim 1, so as to obtain a compound of formula (I) and, optionally converting it into another compound of formula (I) and/or into a pharmaceutically acceptable salt thereof.

5. A process according to claim 4 wherein, in step (b), basic conditions are obtained by means of pyrrolidine.

6. A compound of formula (IIIa) below



(IIIa)

wherein R_1 is a hydrogen atom or a group $-(CH_2)_2-CO_2H$, $-CO_2H$, $-CO_2CH_2CH_3$ and $-CONH-(CH_2)_2-N(CH_2CH_3)_2$.

7. Use of a compound of formula (I), as defined in claim 1, for absorption, distribution, metabolism and excretion (ADME) studies.

ABSTRACT

Compounds which are isotopically labelled carbonium 14 [^{14}C] indolinone derivatives and process for their preparation are disclosed; these compounds are useful for absorption, distribution, metabolism and excretion (ADME) studies.

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